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Acrapex azumai Sugi (Lepidoptera, Noctuidae) as a possible biological control agent of the invasive weed *Imperata cylindrica* (L.) Beauv. (Poaceae) in the United States

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Abstract A field survey was conducted to seek potential biological control agents of *Imperata cylindrica* (L.) Beauv. (Poaceae), which was introduced from Japan and the Philippines into the southeastern USA, and became a highly serious weed there. Lepidopteran larvae were discovered boring in the basal stems of *I. cylindrica* in Itoshima city, Fukuoka Prefecture, Kyushu, Japan in 2013. Adults reared from these larvae were identified as *Acrapex azumai* Sugi (Lepidoptera: Noctuidae). This is the first host record for *A. azumai*. Sequencing of the *COI* (cytochrome oxidase I) gene revealed significant similarity to other *Acrapex* spp., for which comparative sequence data is available. *Acrapex azumai* may have potential as a biological control agent of *I. cylindrica* in the southeastern USA.

Key words biological control, cogongrass, *Imperata cylindrica*, stem borers.

Introduction

The genus *Acrapex* (Noctuidae) includes 83 species and primarily occurs in the Old World, with the majority of species (70) in Africa (Poole, 1989; Hollaway, 1998; Ferguson, 1991). The remaining species are scattered throughout Asia, Australia and Polynesia, and one has been described from North America (Ferguson, 1991). In Japan, *Acrapex azumai* Sugi is the only known representative of the genus, and was first described from adult specimens collected in Okinawa, Japan (Sugi, 1970). Additional adult specimens have been collected in Honshu (Shimane and Chiba Prefectures), Shikoku (Kagawa and Kochi Prefectures), Kyushu (Fukuoka and Kogoshima Prefectures), and Okinawa Prefecture (Okinawa, Miyako, Ishigaki, Iriomote and Yonaguni Islands) (Sugi, 1970; Eda and Shikata, 2011). There are no host records for larvae of *A. azumai*. However, all *Acrapex* spp. for which there are host records, are stem borers in grasses (Swezey, 1927; Le Rü *et al.*, 2006).

Imperata cylindrica (L.) Beauv. (Poaceae) is one of the world's worst weeds, infesting over 500 million ha. in 73 countries (Holm *et al.*, 1977; McDonald, 2009). It is widely distributed in the Old World, with speculation of both Asian (Hubbard, 1944; Gabel, 1982) and African (Evans, 1990; Gabel, 1982) centers of origin. *Imperata cylindrica* is considered native in Japan and two varieties have been described; var. *genuina* in Hokkaido, the northern part of Tohoku District and the highland regions of Central Japan, and var. *koenigii* in the southern parts of Tohoku District and southwards (Tominaga *et al.*, 1989). The distribution of *I. cylindrica* expanded to the New World in the early 1900s when it was introduced from Japan and the Philippines into the southeastern USA (Dozier *et al.*, 1998). Since arriving in the USA, *I. cylindrica* has become a highly invasive weed in Alabama, Mississippi, and Florida, and is a federally listed noxious species (USDA APHIS, 2012). In the course of a field survey to seek potential biological control agents of *I. cylindrica* in Japan, we found *A. azumai* larvae boring and feeding on the stems of *I. cylindrica*.

Biological control of *I. cylindrica* may be the best option to minimize the impacts of this invasive grass in the southeastern U.S. However, few grasses have been targets for biological control (Goolsby *et al.*, 2009, Moran and Goolsby, 2009), and little is known about which functional groups of insect herbivores may have sufficient specificity and impact as biological control agents. Therefore, a survey of the insect herbivores of *I. cylindrica* was conducted in areas of its suspected Asian native range to learn more about the diversity of herbivores and their field biology.

Materials and methods

A survey to identify potential biological control agents of *I. cylindrica* was conducted in Fukuoka and Saga Prefectures, Kyushu, Japan in late July and early August, 2013. Patches of *I. cylindrica* were located visually while driving along secondary roads. Each patch was inspected for actively feeding insect herbivores and symptoms of insect damage. A total of three lepidopteran stem borer larvae were collected from one patch. One of these larvae was preserved in a vial containing 70 % of ethanol and later used for the DNA analysis described below. The other two larvae were reared with fresh *I. cylindrica* stems at room temperature (23-27˚C). After adult emergence, the genitalia of one male was dissected and examined for identification. Based on the distributional information provided by Tominaga *et al*. (1989), the larvae were collected from *I. cylindrica* var. *koenigii*.

In addition to morphological examination, we also 'barcoded' the insect by sequencing the mitochondrial cytochrome c oxidase subunit I gene (*COI*) to provide an additional character for identification and allow submission of specimen information to GenBank. DNA barcoding, when used alone, has been criticized (e.g. Will *et al*., 2005, Rubinoff *et al*., 2006), but is a useful tool when combined with morphological characters (DeSalle 2006, Miller 2007). DNA was extracted from a two ml sample of a larva using a standard Chelex protocol, heating the sample to 56˚C for one hour then heating to 100˚C for eight minutes (Criscione and Blouin, 2005). The DNA was PCR-amplified using the high-fidelity polymerase Phusion® (New England Biolabs) and primers previously described for insect genetic barcoding (LCO1490 and HCO2198) (Folmer *et al*., 1994). PCR cycling conditions were 98˚C for 30 s, 29 cycles of 98˚C for 45 s, 55˚C for 55 s, and 72˚C for 30 s, with a final elongation cycle at 72˚C for 10 minutes.

PCR product was purified using ExoSap-IT® (Affymetrix) then put into sequencing reactions using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied

Table 1. Accession information for *Acrapex* spp. sequences downloaded from NCBI GenBank for comparison with *Acrapex azumai*.

Accession number	Species	Sequenced Locus
KF394482.1	*Acrapex* sp. ANIC1 voucher CCDB-15856-D10	*COI*
KF390299.1	*Acrapex* sp. ANIC1 voucher CCDB-15856-C12	*COI*
KF389783.1	*Acrapex* sp. ANIC6 voucher CCDB-15856-D07	*COI*
KF389678.1	*Acrapex* exsanguis voucher CCDB-15856-D04	*COI*
JX282424.1	*Acrapex syscia**	*COI*
KJ409657	*Acrapex azumai*	*COI*

*According to B. P. Le Rü, who submitted the accession labeled *Acrapex syscia* to GenBank, the identification was incorrect and actually represents an unidentified *Acrapex* sp.

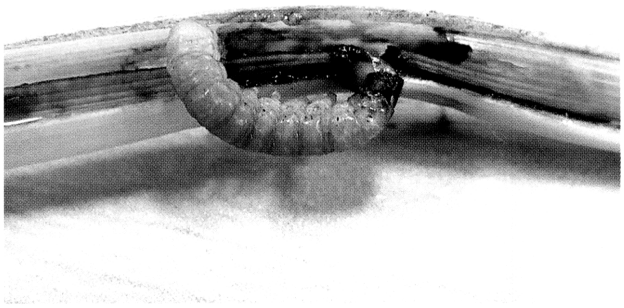

Fig. 1. *Acrapex azumai* larva with a damaged *I. cylindrica* stem.

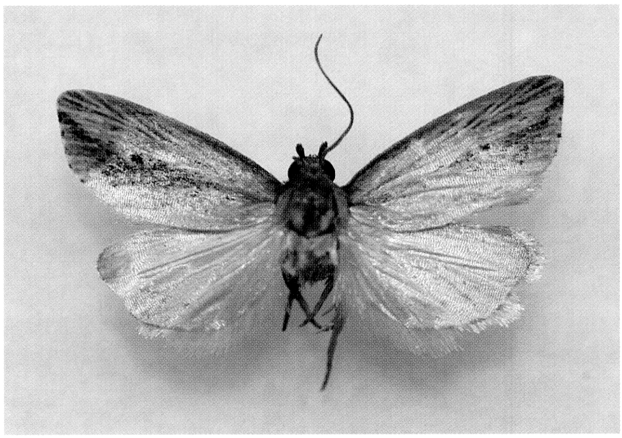

Fig. 2. Adult male *A. azumai*, abdomen removed (forewing length: 8.5 mm, wing span: 18.0 mm).

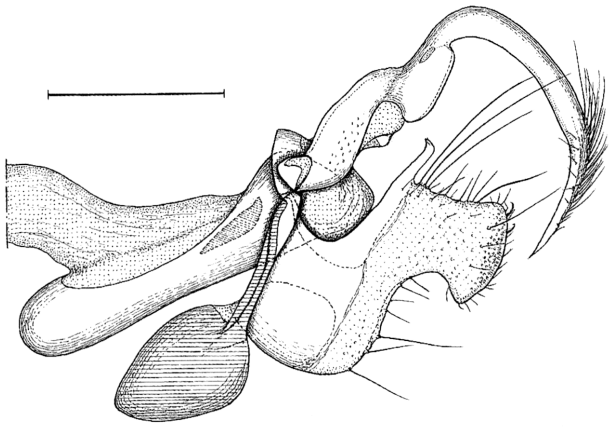

Fig. 3. Male genitalia of *A. azumai*, lateral view. Scale: 0.5 mm.

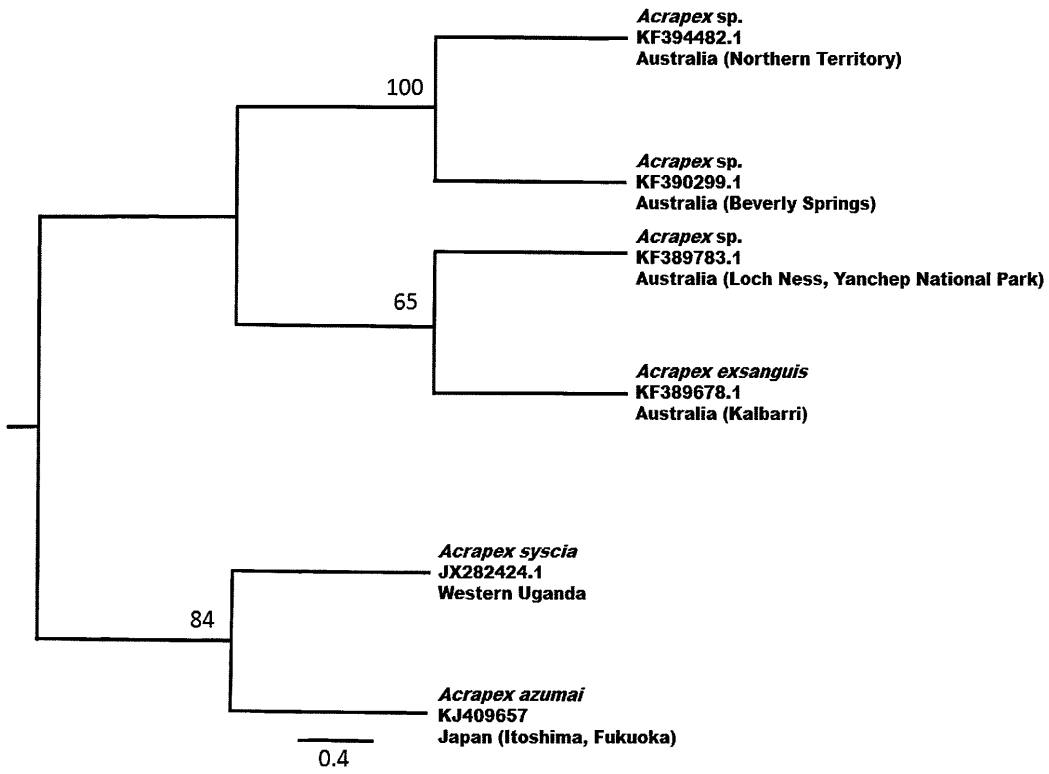

Fig. 4. Phylogenetic relationship of *Acrapex* spp. in the NCBI GenBank using aligned sequence data. Percentage bootstrap values from 1000 replicates are shown. The scale bar indicates branch length, expressed as the expected number of substitutions per site. Location data for all species except *Acrapex syscia* from Uganda are from Hebert *et al.* (2013).

Biosystems). Amplicons were sequenced from both ends on an ABI 3130xl Genetic Analyzer (Applied Biosystems). Primer end sequences were removed from the sequences using Sequencher 4.8 software (GeneCodes). The sequences were aligned with *Acrapex* spp. *COI* data downloaded from NCBI GenBank for comparison (Table 1; Benson *et al.*, 2005). A Neighbor-Joining phylogenetic tree was created using MEGA v. 5.2 (Tamura *et al.*, 2011) software and then rendered in FigTree v. 1.4.0 (Rambaut, 2007).

Results and discussion

Several *I. cylindrica* plants with dead or dying central shoots were observed at one location in Nijo, Itoshima City, Fukuoka Prefecture (N33°49′40″, W130°04′82″). This symptom is often referred to as 'dead heart' in crop grasses, and is typically caused by stem boring insects. Plants expressing this symptom were removed from the soil and carefully inspected. In each case, evidence of longitudinal boring in the basal stem was found, and one lepidopteran larva (~ 1.5 cm in length) was found inside the base of each of three stems (Fig. 1). Among the three larvae collected, two were reared to the adult stage on *I. cylindrica* stems. The larvae pupated on August 12 and 15, 2013, and the adults emerged on August 30 and September 2, 2013.

The wing markings of the two emerged male adults were consistent with *Acrapex azumai* Sugi (1970) (Fig. 2), although their wings (wing span: 18.0 and 19.5 mm) were slightly smaller than the specimens in the original description (wing span: 21-24 mm) (Sugi, 1970). In addition to the wing markings, the male genitalia (Fig. 3) were identical to the descriptions of *A. azumai* in Sugi (1970) as follows: uncus well developed, strongly curved ventrally, ending to acute apex; valva rather short with costa strongly incurved to a free arm, with cucullus strongly bent ventrad with some long setae and a group of short setae on apical inner surface; vesica of aedeagus with a stout thorn-like cornutus together with a pair of tufts of specialized setae latero-apically. Therefore, the adults were identified as *A. azumai*.

The significant match similarity in *COI* barcode fragment supports that this taxon is a member of the genus *Acrapex*. However, it is crucial to note that *COI* often lacks sufficient resolution to delineate at the species level, as is the case with this taxon (Park *et al.*, 2011) (Fig. 4). Bootstrap percentages reveal that *Acrapex azumai* is most closely

related to *Acrapex syscia* collected in western Uganda by B. P. Le Rü (Toussaint *et al.*, 2012). However, according to B. P. Le Rü, who submitted the accession labeled *Acrapex syscia* to GenBank, the identification was incorrect and actually represents an undescribed *Acrapex* sp.

As far as we know, there are no previous host records for *A. azumai*. The feeding habits of very few *Acrapex* have been described, but previous records identify all as stem borers of gramineous plants (Swezey, 1927; Le Rü *et al.*, 2006). Based on ovipositor morphology, Ferguson (1991) suggested that all *Acrapex* shared a similar stem boring feeding behavior. *Acrapex azumai* provides one more example of the larval stem boring behavior of members of the genus, and because of its specialized feeding habit, is a candidate as a biological control agent of *I. cylindrica*. Further study is required to examine its life history, host range and potential impacts in the intended area of introduction before its release for biological control against *I. cylindrica* in the southeastern USA.

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摘　要

米国に侵入した侵入雑草チガヤの生物的防除素材としての可能性を持つチビウスキヨトウ（高須啓志・吉安裕・A. M. Burrell・P. E. Klein・A. Racelis・J. A. Goosby・W. A. Overholt）

米国東南部で大きな被害を出している侵入雑草チガヤ *Imperata cylindrica* (L.)（イネ科）の有効な導入天敵を探索するため，日本においてチガヤの食植性昆虫の探索を行った．2003年7月，福岡県糸島市においてチガヤ茎基部に潜む鱗翅目幼虫を3個体発見した．採集した2個体の幼虫を室内で飼育し，2個体の成虫を得た．成虫は外部形態および交尾器からチビウスキヨトウ *Acrapex azumai* Sugi（鱗翅目：ヤガ科）と同定した．チビウスキヨトウの幼虫の寄主植物はこれまで報告されていないが，本研究で本種の寄主が初めて明らかになった．また，同時に採集した幼虫のミトコンドリア *COI* 遺伝子をGenBankに登録されている *Acrapex* 属の他種と比較したところ，同属の他種と極めて類似性が高いことがわかった．チビウスキヨトウは，米国東南部のチヤガの生物的防除に利用する導入天敵として利用できる可能性がある．

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Acrapex_sp._KF394482.1                TGATCAAATTTATAATACTATTGTTACAGCACATGCTTTTATCATAATTT
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Acrapex_azumai_KJ409657               TGATCAAATTTATAACACTATTGTTACAGCTCATGCTTTTATTATAATTT

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Acrapex_sp._KF392439.1                ----------------------------------------------------
Acrapex_exsanguis_KF389678.1          CAATTATTAATATACGATTAAATAATTTATCTTTTGATCAAATACCTTTA
Acrapex_syscia_JX282424.1             CAATTATTAATATACGATTAAATAACCTATCTTTTGATCAAATACCTTTA
Acrapex_azumai_KJ409657               CAATTATTAATATACGATTAAATAGTTTATCCTTTGATCAAATACCTTTA


Acrapex_sp._KF394482.1                TTTATTTGAGCTGTTGGAATTACTGCATTTCTATTATTACTTTCATTA--
Acrapex_sp._KF390299.1                TTTATTTGAGCTGTTGGAATTACTGCATTTCTATTATTACTTTCATTA--
Acrapex_sp._KF389783.1                TTTATTTGAGCTGTTGGAATTACTGCATTTTTACTATTACTTTCATTACC
Acrapex_sp._KF392439.1                ----------------------------------------------------
Acrapex_exsanguis_KF389678.1          TTTGTTTGAGCTGTTGGAATTACTGCATTTTTACTATTACTCTCATTA--
Acrapex_syscia_JX282424.1             TTTATTTGAGCTGTAGGTATCACCGCATTCTTACTATTATTATCTTTACC
Acrapex_azumai_KJ409657               TTTATTTGAGCTGTAGGAATTACTGCATTTTTATTATTACTTTCTTTACC

                                                  *       *
Acrapex_sp._KF394482.1                ----------------------------------------------
Acrapex_sp._KF390299.1                ----------------------------------------------
Acrapex_sp._KF389783.1                TGTATTAGCTGGAGCTATTAC-AATATTATTAACTGATCGAAA
Acrapex_sp._KF392439.1                ----------------------------------------------
Acrapex_exsanguis_KF389678.1          ----------------------------------------------
Acrapex_syscia_JX282424.1             TGTTTTAGCTGGAGCTATTAC-TATACTATTAACAGATCGAAA
Acrapex_azumai_KJ409657               TGTATTAGCTGGAGCTATTACCAATATTACTAACAGATCGAAA
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Supplemental Figure 1. Nexus formatted sequence data of *Acrapex* spp. based on 205-493 bp of *COI*. Asterisks demarcate single nucleotide polymorphisms in *A. azumai* in comparison to other *Acrapex* species for which sequence data was available. Note that accession no. KF392439.1 was not included in the phylogenetic analysis depicted in figure 4 because the length of the sequenced fragment was too short and therefore would have biased the results